

TITRATION CURVES OF CERTAIN LIQUID CULTURE MEDIA

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A study of the growth of various fungi and of the germination of various fungous spores with reference to H-ion concentration has involved a determination of the titration curves of several culture media. It is the purpose of this paper to present briefly the data obtained with these nutrient solutions, which have been the basis of subsequent work in this laboratory.

The formulae for the media employed are as follows:

Beet decoction.—Prepared according to the method outlined by Duggar, Severy, and Schmitz ('17). This consists essentially of 370.4 gms. of sugar beets per liter of distilled water, autoclaved at 15 pounds for one hour, and then filtered.

Czapek's solution.— $\text{MgSO}_4 + 7 \text{H}_2\text{O}$, .5 gm.; KH_2PO_4 , 1.0 gm.; KCl , .5 gm.; NaNO_3 , 2.0 gms.; FeSO_4 , .01 gm.; cane sugar, 30.0 gms.; distilled H_2O , 1000 cc. (Zeller, Schmitz, and Duggar, '19).

Peptone solution.—Twenty gms. bacto-peptone in 1000 cc. H_2O .

Pfeffer's solution.— KH_2PO_4 , 5.0 gms.; $\text{MgSO}_4 + 7 \text{H}_2\text{O}$, 2.5 gms.; NH_4NO_3 , 10.0 gms.; FeSO_4 , trace; cane sugar, 50.0 gms.; distilled H_2O , 1000 cc. (Pfeffer, '95).

Richards' solution.— KH_2PO_4 , .5 gm.; KNO_3 , 4.0 gms.; $\text{MgSO}_4 + 7 \text{H}_2\text{O}$, .5 gm.; NH_4NO_3 , 10.0 gms.; FeSO_4 , trace; cane sugar, 30.0 gms.; distilled H_2O , 1000 cc. (Richards, '97). This formula differs from that of the original by a reduction in the amount of MgSO_4 . The amount of precipitate produced in the more alkaline solutions was found to depend largely upon the amount of magnesium present, and, since it is important to keep the amounts of the constituents in the solutions as nearly equal as possible while varying the H-ion concentration, it seemed desirable to reduce the amount of MgSO_4 to .5 gm. per liter. Such

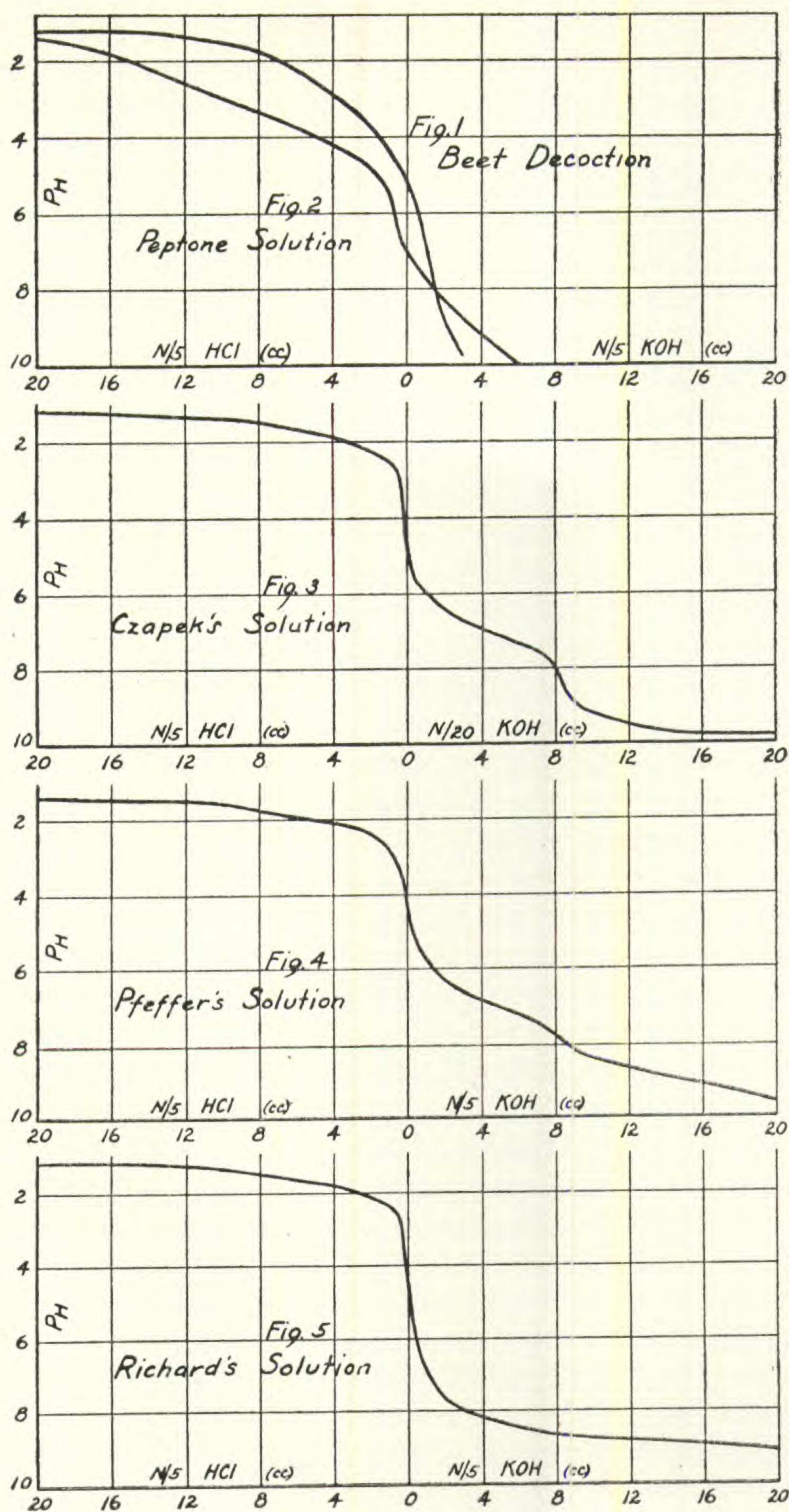
a reduction has been found by Duggar (unpublished data) to have no appreciable effect upon the growth of fungi.

The nutrient solutions were all prepared by adding 600 cc., instead of 1000 cc., of distilled water. This method allowed dilutions of the various solutions by additions of regulated amounts of acid or alkali and water for the adjustment of various H-ion concentrations without affecting materially the concentrations of the nutrient salts or constituents.

It is possible to obtain a wide range in the H-ion concentrations of the solutions by adding an alkali and a mineral acid in successively increasing quantities. The addition of the mono-, the di-, or the tri-basic potassium phosphate as suggested by Zeller, Schmitz, and Duggar ('19) was found to give results within a certain range, but additions in too large amounts were necessary to produce extreme concentrations. Therefore, since such a decided variation in the composition of the nutrient solutions undoubtedly would have existed, it seemed undesirable to adopt this method in the experiments under investigation.

From preliminary experimentation relative to the adjustment of H-ion concentration in media of alkaline reactions, the most satisfactory results were obtained by using N/5 KOH, and an alkali of this strength has been employed in all of the experiments except in the case of Czapek's solution. Here, the buffer action was less than that in the other nutrient solutions, consequently N/20 concentration was more conveniently and accurately applicable. In all the experiments, however, N/5 HCl was favorable for varying the reactions on the acid side. In the case of Czapek's solution, the reactions were also varied by means of N/1 H_3PO_4 and N/20 NaOH. These results so nearly paralleled those obtained with the above acid and alkali that it was deemed unnecessary to continue this aspect of the experiment with the other nutrient solutions.

Inasmuch as it is generally admitted that sugars readily react with acid or alkali when heated under pressure, the nutrient solutions and the acid and the alkali were sterilized separately. Thirty cc. of the nutrient solution together with the desired amount of water, as indicated in the tables, were put into small flasks, plugged, and sterilized. After cooling, the



cultures were removed to a culture chamber, and definite amounts of sterile acid and alkali were added by means of sterile graduated pipettes. The final volume of each culture was 50 cc. and represented a dilution of the constituents comparable with that in the original nutrient solutions. The solutions were allowed to stand for 24 hours in order to reach a state of equilibrium, and, at the expiration of this period, H-ion determinations were made according to the colorimetric method of Clark and Lubs ('17), all determinations being made at room temperature. (See figs. 1-5 and tables I-II).

Due to the presence of color in the beet decoction and in the peptone solution, it was necessary to use a colorimeter for the H-ion determinations. A Duboscq type was used in this particular work, the detailed method of which has been described by Duggar ('19).

In all of the mineral salt solutions, a certain amount of precipitate occurred upon the addition of alkali, the amount increasing with increase of added alkali. No such phenomenon was evidenced in the alkaline cultures of the beet decoction or of the peptone solution. The precipitation referred to commenced with culture No. 29 in Pfeffer's solution, No. 27 in Richards' solution, and very faintly in No. 23 of Czapek's solution. On the other hand, there was no precipitation whatever in the acid cultures of any of the various media.

As the reaction of the beet decoction passed from acid to alkaline, there was noted a decided color change from pale yellow to amber, and a slight cloudiness was perceptible beginning with culture No. 42.

The titration curve obtained with 2 per cent bacto-peptone agrees closely with the curves obtained by Clark and Lubs ('17) with Witte peptone, falling, as one would expect, between the curves representing concentrations of 1 and 5 per cent.

The initial P_H of the original culture solutions varied, variations of several tenths not being infrequent despite the most careful technique during preparation and the use of highest purity chemicals. Since the highest purity mono-basic potassium phosphate obtainable had a very high acidity due to the impurities present, the salt was recrystallized until the Sørensen coefficient of P_H 4.529 for a 1/15 molecular solution was obtained.

TABLE I
TITRATION DATA FOR VARIOUS LIQUID MEDIA

No.	Solution (cc.)	N/5 HCl (cc.)	N/5 KOH (cc.)	Dist. H ₂ O (cc.)	Total (cc.)	Hydrogen ion concentration, P _H			
						Beet decoc- tion	Rich- ards	Pep- tone	Pfef- fer
1	30	20.00		00.00	50	1.2	1.2	1.4	1.4
2	30	15.00		05.00	50	1.2	1.2	2.0	1.5
3	30	10.00		10.00	50	1.6	1.4	3.0	1.5
4	30	9.50		10.50	50		1.4		1.6
5	30	9.00		11.00	50	1.7	1.5	3.2	1.7
6	30	8.50		11.50	50		1.5		1.7
7	30	8.00		12.00	50	1.9	1.5	3.4	1.8
8	30	7.50		12.50	50		1.6		1.8
9	30	7.00		13.00	50	2.1	1.6	3.6	1.9
10	30	6.50		13.50	50		1.6		1.9
11	30	6.00		14.00	50	2.3	1.7	3.8	1.9
12	30	5.50		14.50	50		1.7		2.0
13	30	5.00		15.00	50	2.6	1.7	4.0	2.1
14	30	4.50		15.50	50		1.8		2.1
15	30	4.00		16.00	50	3.1	1.8	4.2	2.1
16	30	3.50		16.50	50		2.0		2.3
17	30	3.00		17.00	50	3.4	2.0	4.4	2.3
18	30	2.50		17.50	50	3.6			2.3
19	30	2.00		18.00	50	3.8	2.2	4.9	2.4
20	30	1.50		18.50	50				2.7
21	30	1.00		19.00	50	4.4	2.4	5.4	2.8
22	30	.75		19.25	50	4.5			
23	30	.50		19.50	50	4.8	2.8	6.4	3.2
24	30	.25		19.75	50	5.0			
25	30	0.00		20.00	50	5.2	4.6	7.0	4.3
26	30		.25	19.75	50	5.4			
27	30		.50	19.50	50	5.6		7.4	5.3
28	30		.75	19.25	50	6.4			
29	30		1.00	19.00	50	7.0	7.0	7.8	5.6
30	30		1.50	18.50	50		7.5		6.2
31	30		2.00	18.00	50	8.8	7.7	8.2	6.3
32	30		2.50	17.50	50		7.9		6.4
33	30		3.00	17.00	50	9.8	8.1	8.8	6.6
34	30		3.50	16.50	50		8.2		6.6
35	30		4.00	16.00	50	10.0+	8.2	9.2	6.8
36	30		4.50	15.50	50		8.3		6.9
37	30		5.00	15.00	50		8.3	9.6	7.0
38	30		5.50	14.50	50		8.4		7.1
39	30		6.00	14.00	50		8.5	10.0	7.2
40	30		6.50	13.50	50		8.6		7.3
41	30		7.00	13.00	50		8.6	10.0	7.4
42	30		7.50	12.50	50		8.7		7.6
43	30		8.00	12.00	50		8.7	10.0+	7.8
44	30		8.50	11.50	50		8.7		8.0
45	30		9.00	11.00	50		8.7	10.0+	8.2
46	30		9.50	10.50	50		8.7		8.3
47	30		10.00	10.00	50		8.8	10.0+	8.4
48	30		15.00	5.00	50		8.9		9.0
49	30		20.00	0.00	50		9.1		9.6

TABLE II
TITRATION DATA FOR CZAPEK'S SOLUTION

No.	Czapek sol. (cc.)	Acid (cc.)	Alkali (cc.)	Dist. H ₂ O (cc.)	Total cc.	H-ion concentration, P _H	
						N/5 HCl N/20 KOH	N/1 H ₃ PO ₄ N/20 NaOH
1	30	20.00		0.00	50	1.2	
2	30	15.00		5.00	50	1.3	
3	30	10.00		10.00	50	1.4	1.6
4	30	9.50		10.50	50	1.4	1.6
5	30	9.00		11.00	50	1.5	1.6
6	30	8.50		11.50	50	1.5	1.7
7	30	8.00		12.00	50	1.5	1.7
8	30	7.50		12.50	50	1.6	1.7
9	30	7.00		13.00	50	1.6	1.7
10	30	6.50		13.50	50	1.6	1.8
11	30	6.00		14.00	50	1.7	1.8
12	30	5.50		14.50	50	1.7	1.8
13	30	5.00		15.00	50	1.8	1.9
14	30	4.50		15.50	50	1.9	1.9
15	30	4.00		16.00	50	1.9	2.0
16	30	3.50		16.50	50	2.0	2.0
17	30	3.00		17.00	50	2.1	2.1
18	30	2.50		17.50	50	2.2	2.2
19	30	2.00		18.00	50	2.3	2.3
20	30	1.50		18.50	50	2.4	2.3
21	30	1.00		19.00	50	2.5	2.4
22	30	.50		19.50	50	2.9	2.6
23	30	0.00		20.00	50	4.7	4.4
24	30		.50	19.50	50	5.7	5.6
25	30		1.00	19.00	50	6.0	5.9
26	30		1.50	18.50	50	6.2	6.2
27	30		2.00	18.00	50	6.4	6.3
28	30		2.50	17.50	50	6.6	6.5
29	30		3.00	17.00	50	6.7	6.6
30	30		3.50	16.50	50	6.8	6.8
31	30		4.00	16.00	50	6.9	6.8
32	30		4.50	15.50	50	7.0	7.0
33	30		5.00	15.00	50	7.1	7.1
34	30		5.50	14.50	50	7.2	7.2
35	30		6.00	14.00	50	7.4	7.4
36	30		6.50	13.50	50	7.5	7.6
37	30		7.00	13.00	50	7.6	7.7
38	30		7.50	12.50	50	7.7	7.9
39	30		8.00	12.00	50	8.0	8.2
40	30		8.50	11.50	50	8.6	8.6
41	30		9.00	11.00	50	8.9	8.7
42	30		9.50	10.50	50	9.1	9.0
43	30		10.00	10.00	50	9.2	9.2
44	30		15.00	5.00	50	9.8	
45	30		20.00	0.00	50	9.8	

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